Remarks

Claims 1, 4-9, 12-13 and 15-16 are pending after entry of the amendments set forth herein. Claims 2-3, 10-11 and 14 are canceled without prejudice. Claim 1 is amended, and new Claims 15-16 are added. Support for these amendments is found in the specification at paragraph 55. No new matter is added. Reconsideration is requested.

Rejections Under §112, ¶1

Claims 1 and 5 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. With respect to the claim limitation "give rise exclusively to megakaryocyte colonies", the Office Action states that "there is no support, either explicit or otherwise, for this limitation in the specification."

Applicants respectfully submit that the specification supports the amending language. As noted by Applicants in the response of August 7, 2007, at paragraph 11, line 8 of the specification it is stated that "MEPs gave rise to both megakaryocyte and erythroid colonies while MKPs formed exclusively megakaryocyte colonies".

The Examiner references the specification at paragraph 79, which cite if taken in its entirety states:

> MKPs exclusively form megakaryocyte colonies *in vitro*. In the methycellulose culture system that was set up to detect all possible outcomes of myeloid differentiation, MKPs gave rise mainly to CFU-MK with less than 1% CFU-GM, CFU-M and BFU-E (Table 1). The readouts of these non-megakaryocyte colonies can be totally eliminated by using more restricted sorting gates, indicating that they were derived from the contaminants from the other progenitor pools rather than from MKPs. Interestingly, about 1-2% of MKP-derived colonies were large colonies that resembled the burst-forming unit-megakaryocyte (BFU-MK) previously described by Long *et. al.* (1985) *J. Clin. Invest.* 76, 431-438. The morphology of MKP-derived CFU-MK and BFU-MK are shown in Figure 2A. Giemsa staining of cells in the individual colonies revealed that CFU-MK contained only mature megakaryocytes (Figure 2B) whereas BFU-MK also frequently contained small numbers of erythroid cells. Therefore, these colonies should be more accurately defined as BFU-MK/E and probably represent the earlier stage of megakaryocyte progenitors.

Applicants note that the data provided in the application provides "less than 1% CFU-GM, CFU-M and BFU-E (Table 1). The readouts of these non-megakaryocyte colonies can be totally eliminated by using more restricted sorting gates". Thus, only a tiny fraction of cells give rise to colonies other than megakaryocyte colonies, and if the sorting gates, i.e. the parameters used for cell selection, are made more restrictive, then the contaminants are eliminated.

It appears that the Examiner is questioning Applicants' data – data which was published in the journal PNAS (copy attached) and which has been widely cited in a number of scientific articles, including:

- K. Kaushansky Blood, February 1, 2008; 111(3): 981 – 986, "Historical review: megakaryopoiesis and thrombopoiesis"
- K. Matsumura-Takeda et al. Stem Cells, April 1, 2007; 25(4): 862 – 870, "CD41+/CD45+ Cells Without Acetylcholinesterase Activity Are Immature and a Major Megakaryocytic Population in Murine Bone Marrow"
- B. Ghinassi et al. Blood, February 15, 2007; 109(4): 1460 – 1471, "The hypomorphic Gata1low mutation alters the proliferation/differentiation potential of the common megakaryocytic-erythroid progenitor"
- J. Tober et al. Blood, February 15, 2007; 109(4): 1433 – 1441, "The megakaryocyte lineage originates from hemangioblast precursors and is an integral component both of primitive and of definitive hematopoiesis"
- T. G.P. Bumm et al. Cancer Res., December 1, 2006; 66(23): 11156 - 11165." Characterization of Murine JAK2V617F-Positive Myeloproliferative Disease"
- K. Guo et al. Cancer Res., October 1, 2006; 66(19): 9625 – 9635, "PRL-3 Initiates Tumor Angiogenesis by Recruiting Endothelial Cells In vitro and In vivo"
- .Y. Lai et al. J. Exp. Med., August 7, 2006; 203(8): 1867 – 1873, "Asymmetrical lymphoid and myeloid lineage commitment in multipotent hematopoietic progenitors"
- Kim et al. Blood, July 15, 2006; 108(2): 737 – 744, "Enhanced purification of fetal liver hematopoietic stem cells using SLAM family receptors"
- D. L. Stachura et al. Blood, January 1, 2006; 107(1): 87 – 97, "Early block to erythromegakaryocytic development conferred by loss of transcription factor GATA-1"
- H.-F. Lin et al. Blood, December 1, 2005; 106(12): 3803 – 3810, "Analysis of thrombocyte development in CD41-GFP transgenic zebrafish"
- Kuhl et al. Mol. Cell. Biol., October 1, 2005; 25(19): 8592 - 8606
- GATA1-Mediated Megakaryocyte Differentiation and Growth Control Can Be Uncoupled and Mapped to Different Domains in GATA1
- Weissman JAMA, September 21, 2005; 294(11): 1359 – 1366, "Stem Cell Research: Paths to Cancer Therapies and Regenerative Medicine"
- W. B. Slayton et al. Stem Cells, September 1, 2005; 23(9): 1400 – 1408, "Developmental Differences in Megakaryocyte Maturation Are Determined by the Microenvironment"
- C.-C. Chen et al. PNAS, August 9, 2005; 102(32): 11408 – 11413, "From The Cover: Identification of mast cell progenitors in adult mice"
- .J. R. Gothert et al. Blood, April 1, 2005; 105(7): 2724 – 2732, "In vivo fate-tracing studies using the Scl stem cell enhancer: embryonic hematopoietic stem cells significantly contribute to adult hematopoiesis"
- G. Terszowski et al. Blood, March 1, 2005; 105(5): 1937 – 1945, "Prospective isolation and global gene expression analysis of the erythrocyte colony-forming unit (CFU-E)"
- Metcalf, et al. Stem Cells, January 1, 2005; 23(1): 55 – 62, "Murine Megakaryocyte Progenitor Cells and Their Susceptibility to Suppression by G-CSF"
- Takahashi, et al. Cancer Res., December 15, 2004; 64(24): 8839 – 8845, "Evidence for the Involvement of Double-Strand Breaks in Heat-Induced Cell Killing"
- H. L. Bradley et al. Blood, April 15, 2004; 103(8): 2965 – 2972, "Hematopoietic-repopulating defects from STAT5-deficient bone marrow are not fully accounted for by loss of thrombopoietin responsiveness"
- H. Takano et al. J. Exp. Med., February 2, 2004; 199(3): 295 – 302, "Asymmetric Division and Lineage Commitment at the Level of Hematopoietic Stem Cells: Inference from Differentiation in Daughter Cell and Granddaughter Cell Pairs"

- S. Huettner et al. Blood, November 1, 2003; 102(9): 3363 – 3370, "Inducible expression of BCR/ABL using human CD34 regulatory elements results in a megakaryocytic myeloproliferative syndrome".

Applicants respectfully submit that the Examiner has provided no reason to doubt the scientific veracity of Applicants findings, which have been both published and widely cited in the scientific literature. In view of the above amendments and remarks, withdrawal of the rejection is requested.

Rejections Under §102

Claims 1, 4, 7, and 8 remain rejected under 35 U.S.C. 102(b) as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Clay et al. (2001, *Blood* 97: 1982-1989). Applicants respectfully submit that the presently claimed invention is not anticipated or suggested by the cited art.

The present claims were amended to recite a cell that exclusively gives rise to megakaryocytes and progenitors, and are further amended to recite a therapeutic composition, which may be frozen (as set forth in Claim 15) or have at least 10^6 cells present (as set forth in Claim 16).

As previously discussed by Applicants in an earlier response, as further explicated by Dr. Karsunky in the previously submitted Declaration, and as described above, the presently claimed invention is directed to a megakaryocyte progenitor cell population that only gives rise to megakaryocyte colonies.

The cells isolated by Clay *et al.* differ from the presently claimed cells in their cell surface phenotype and in their developmental potential. On page 1985 of Clay et al., it is stated that "in some experiments, $CD9^+CD41^+$ cells were sorted according to CD41 expression; gates D and C (Figure 3) corresponded to $CD41^{mid/low}$ and $CD41^{high}$ cells, respectively."

Clay *et al.* analyzed the sorted cells as follows:

> In contrast to the other myeloid progenitors, when $CD34^+CD41^-$ bone marrow cells were sorted into $CD9^{low}$, $CD9^{mid}$, and $CD9^{high}$ subpopulations, CFU-MK were highly enriched in the $CD9^{high}$ population (Figures 4, 5). Only a small proportion of CFU-MK was detected in the $CD9^{low}$ fraction, and an intermediate proportion was detected in the $CD9^{mid}$ fraction. As indicated earlier, a small fraction of the $CD34^+CD9^+$ cells expresses the CD41 antigen. Therefore, we sorted $CD9^{high}$ and $CD9_{mid}$ cells according to their CD41 expression level (Figure 3). The proportion of CFU-MK was 4-fold higher in the $CD9^{mid}CD41^{neg}$ fraction (gate B) than in the $CD9^{mid}CD41^{mid/low}$ population (gate D) (24 ± 2 and 6 ± 1, respectively, for 10_4 plated cells). In contrast, $CD9^{high}CD41^{high}$ (gate E) only gave rise to a small number of differentiated megakaryocytic clusters.

When EPO was added to these cultures, BFU-E/MK mainly arose from the differentiation of the $CD9^{mid}$ cells (21 ± 16 for 10^4 plated cells) compared to $CD9^{high}$ (7 ± 4 for 10^4 plated cells). Few BFU-E/MK were detected in the $CD9^{low}$ fraction and even then only in some experiments (data not shown).

One of skill in the art will conclude from the teachings of Clay *et al.* that the cell population of $CD34^+CD41^+CD9^+$ cells that are unsorted for lineage markers comprises progenitor cells for mixed erythroid colonies (BFU-E/MK), and therefore is not a population that gives rise exclusively to megakaryocytes and platelets.

Applicants submit that the phenotypic and functional characterization of the prior art cell population differs from the characteristics of the presently claimed cell population. One of skill in the art would not be motivated to pursue a monopotent megakaryocyte progenitor cell in the cell populations defined by Clay *et al.*, as the $CD34^+CD41^+CD9^+$ population was stated to contain only a small number of differentiated megakaryocyte clusters.

Clay *et al.* further fail to teach a use for the cells of the present claims. The therapeutic use of the cells described in the instant specification relates to their special role as a dedicated, monopotent megakaryocyte progenitor, and could not have been predicted by the cited art.

The claim limitations relating to the dose of cells, to the use of a physiologically acceptable medium, and to the freezing of the cells for future therapeutic use rely on the teachings of the present application, and are not taught or suggested by the cited art. In view of the above amendments and remarks, withdrawal of the rejection is requested.

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number STAN-278.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: June 13, 2008

By: _____
Pamela J. Sherwood, Ph.D.
Registration No. 36,677

BOZICEVIC, FIELD & FRANCIS LLP
1900 University Avenue, Suite 200
East Palo Alto, California 94303
Telephone: (650) 327-3400
Facsimile: (650) 327-3231